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Potential cardiovascular disease risk markers among HIV-infected women initiating antiretroviral treatment

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Abstract

Background—Inflammation and hemostasis perturbation may be involved in vascular complications of HIV infection. We examined atherogenic biomarkers and subclinical atherosclerosis in HIV-infected adults before and after beginning highly-active antiretroviral therapy (HAART).

Methods—In the Women's Interagency HIV Study (WIHS), 127 HIV-infected women studied pre- and post-HAART were matched to HIV-uninfected controls. Six semi-annual measurements of soluble CD14, tumor necrosis factor (TNF)-alpha, soluble interleukin (IL)-2 receptor, IL-6, IL-10, monocyte chemoattractant protein (MCP)-1, D-dimer, and fibrinogen were obtained. Carotid artery intima-media thickness (CIMT) was measured by B-mode ultrasound.

Results—Relative to HIV-uninfected controls, HAART-naïve HIV-infected women had elevated levels of soluble CD14 (1945 vs 1662 ng/mL, Wilcoxon signed rank $P<0.0001$), TNF-alpha (6.3 vs 3.4 pg/mL, $P<0.0001$), soluble IL-2 receptor (1587 vs 949 pg/mL, $P<0.0001$), IL-10 (3.3 vs 1.9 pg/mL, $P<0.0001$), MCP-1 (190 vs 163 pg/mL, $P<0.0001$) and D-dimer (0.43 vs 0.31 μg/mL,

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P<0.01). Elevated biomarker levels declined after HAART. While most biomarkers normalized to HIV-uninfected levels, in women on effective HAART, TNF-alpha levels remained elevated compared to HIV-uninfected women (+0.8 pg/mL, *P*=0.0002). Higher post-HAART levels of soluble IL-2 receptor (*P*=0.02), IL-6 (*P*=0.05), and D-dimer (*P*=0.03) were associated with increased CIMT.

Conclusions—Untreated HIV infection is associated with abnormal hemostasis (e.g., D-dimer), and pro-atherogenic (e.g., TNF-alpha) and anti-atherogenic (e.g., IL-10) inflammatory markers. HAART reduces most inflammatory mediators to HIV-uninfected levels. Increased inflammation and hemostasis are associated with subclinical atherosclerosis in recently treated women. These findings have potential implications for long-term risk of cardiovascular disease in HIV-infected patients, even with effective therapy.

Keywords

antiretroviral therapy; cardiovascular diseases; cytokines; hemostasis; HIV; inflammation

Introduction

Chronic HIV infection is associated with immune activation, inflammation in various tissues, changes in hemostatic balance, and potentially with cardiovascular disease (CVD) risk[1]. Inflammatory and hemostatic biomarkers appear to provide clinically meaningful information about how HIV infection affects the host[2,3]. Further study is important for several reasons. First, treated and untreated HIV-infected individuals, even at high CD4+ T cell counts, appear to have higher risk of mortality as compared with HIV-uninfected individuals, and inflammation remains an important risk factor for death[4–6]. Identification of specific inflammation and coagulation mediators involved in HIV disease would contribute understanding to these mechanisms of comorbidity. Second, biomarkers might supplement those used in clinical practice to predict patient prognosis including future risk of CVD events. Third, biomarkers that can be reproducibly shown to predict complications of HIV infection may suggest modalities of future targeted therapy. Fourth, while prior studies have described inflammation and coagulation biomarkers in HIV-infected women, few have included a group of HIV-uninfected controls who are well-matched for other factors that may influence these markers[7–10]. Finally, serum biomarkers and measures of subclinical atherosclerosis such as carotid artery intima-media thickness (CIMT) are potential endpoints that can be used to examine whether elevated CVD risk might persist even in effectively-treated HIV-infected adults.

Among participants in the Women's Interagency HIV Study (WIHS), we examined the association of HIV infection, and first initiation of highly-active antiretroviral therapy (HAART), with biomarkers of inflammation and hemostasis, including: pro-inflammatory cytokines such as tumor necrosis factor (TNF)-alpha and interleukin (IL)-6; soluble IL-2 receptor; IL-10, an anti-inflammatory Th2 cytokine also produced by monocytes/macrophages and regulatory T cells (T_{regs}); monocyte chemoattractant protein-1 (MCP-1)/CCL2, a chemokine that has been associated with atherosclerosis in the HIV-infected population; soluble CD14, a pathogen recognition receptor expressed by monocytes that predicts CVD events among HIV-infected patients; D-dimer, which is produced during the degradation of the fibrin clot; and fibrinogen, an inflammatory marker that functions as modulator of platelet and coagulation protein activity and is a fibrin precursor. Among women initiating HAART, we further examined the association of biomarker level both prior to and after initiating treatment, with subclinical atherosclerosis.

Methods

Study design and variable definition

The WIHS cohort enrolled 3,766 HIV-infected and HIV-uninfected women who were recruited in two waves (1994–1995 and 2001–2002) at six US field centers. Every six months, WIHS participants are scheduled for study examinations, which involve collection of interview-administered questionnaire data, physical measurements and biospecimens [11,12]. Institutional Review Board approval and informed consent were obtained on all participants.

Using medication questionnaire data collected at each semi-annual visit, we identified 769 HIV-infected women who first reported use of HAART without any prior reported use of antiretroviral therapy while enrolled in WIHS. Of these women, we identified 127 who had provided blood specimens at six consecutive semi-annual WIHS study examinations, including three prior to and three after use of HAART. Compared to the HIV-infected women in WIHS excluded from the study, the 127 women included had a lower median baseline age (33 years versus 35 years). Women were similar in terms of race/ethnicity, smoking status, body mass index (BMI), and hepatitis C virus (HCV) antibody status. Our comparison population consisted of a group of HIV-uninfected women enrolled in WIHS who were individually matched to the HIV-infected women using propensity score matching that accounted for age, race/ethnicity, BMI, smoking status, HCV antibody status, and calendar time[13]. In order to find the best matched HIV-uninfected woman for all 127 of the HIV-infected women who initiated HAART, 29 HIV-uninfected women were selected more than once; for these women, we chose different sequences of six visits that were matched with different HIV-infected HAART initiators. HAART was defined as reported use of combination therapy in accordance with US Department of Health and Human Services guidelines, with major classes defined by cornerstone therapies: protease inhibitors (PIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and nucleoside reverse transcriptase inhibitors (NRTIs).

Carotid artery ultrasound

During 2004–2005, we obtained B-mode ultrasound carotid artery measurements of the intima-media thickness of the far wall of the right common carotid artery (CIMT). Standardized carotid artery ultrasound images were centrally measured by automated computerized edge detection software (Patents, 2005, 2006, 2011)[14–16].

Laboratory values

HIV infection was determined via serologic testing using enzyme-linked immunosorbent assay (ELISA) and confirmed using Western blot assays. Plasma HIV RNA levels were quantified using nucleic acid sequence based amplification commercial assays with a lower limit of quantification of 80 copies/mL (bioMérieux, Boxtel, NC), and total peripheral CD4+ T-cell counts were measured with standard flow cytometric methods. HCV antibody testing was performed using enzyme immunoassays (version 2.0 or 3.0; Abbott, Abbott Park, IL). HCV RNA levels were measured by a polymerase chain reaction (Roche Diagnostics, Indianapolis, IN)[17]. ELISA methods were used to measure soluble IL-2 receptor (DR2A00, R&D Systems, Minneapolis, MN), IL-6 (Q6000B, R&D Systems, Minneapolis, MN) and soluble CD14 (DC140, R&D Systems, Minneapolis, MN). Fibrinogen activity was measured using a clot-based assay (00674) and D-dimer using immunoturbidimetric methods (00515) (Stago Diagnostics, Parsippany, NJ). MCP-1, TNF-alpha and IL-10 were measured on a bead-based immunoassay multiplex platform (MPXHCYTO-60K-04 Cytokine Panel, Millipore Corporation, Billerica, MA). We previously described an

association between HAART initiation and increased C-reactive protein levels in the WIHS cohort, and did not repeat this measurement in the present study[10].

Statistical analyses

We compared characteristics in the HIV-infected and HIV-uninfected women using Chi Square and Mann-Whitney tests, for categorical and continuous variables, respectively. We examined summary statistics of each biomarker and correlations among inflammatory and hemostasis biomarkers. We also examined the association of biomarker levels with current and nadir CD4+ T cell count, HIV RNA, and clinical characteristics including age, HCV antibody status, smoking, and BMI. We computed median biomarker values and Wilcoxon signed rank tests to compare HIV-infected and HIV-uninfected women and to examine changes in biomarkers associated with first use of HAART among the HIV-infected group. Linear mixed-effects models were also used to assess statistical significance of biomarker level comparisons, summarized across all pre-HAART and post-HAART visits. Models incorporated random intercept and slope terms for each individual to account for the within-individual correlation among the six contributed measurements, and heterogeneous rates of change across individuals. Results obtained using the linear mixed-effects models were similar to those from the Wilcoxon signed rank test-based comparisons of the raw data, so are not presented.

After comparing biomarker levels in HIV-infected women prior to treatment and after HAART initiation relative to matched HIV-uninfected women, we repeated analyses while limiting visits among HIV-infected women to those where they were treated and aviremic (HIV RNA below 80 copies/mL). HCV was a common co-infection that may contribute to inflammation or influence production of liver-derived coagulation and inflammation markers; therefore, we used stratified analysis and tests of statistical interaction to examine whether HCV coinfection (HCV RNA+ at study entry) was an effect modifier of the associations of HIV infection status and use of HAART with inflammation and coagulation biomarkers. Finally, we used multivariable linear regression to assess the difference in CIMT in μm associated with a 10% increase in biomarker level, in models that were adjusted for age, race/ethnicity, smoking, BMI and time between biomarker measurements and carotid artery ultrasound visit. Analyses were performed using SAS version 9.2 (Cary, NC).

Results

Subject characteristics

HIV-infected and HIV-uninfected women were both of median age 37 years and were similar on other matching factors (Table 1). In both groups, well over half of women were overweight or obese, approximately half were current smokers, and nearly one-third had detectable HCV antibodies.

Antiretroviral medications

All HIV-infected women initiated HAART while under study observation. The most common antiretroviral medications used were, in the PI class: nelfinavir (used at 16% of treated study visits), indinavir (16%), ritonavir (14%), and atazanavir (11%); in the NNRTI class: efavirenz (20%), nevirapine (16%); in the NRTI class: lamivudine (68%), zidovudine/AZT (44%), tenofovir (25%), emtricitabine (22%), stavudine/d4T (21%), abacavir (11%); and fixed-dose combinations: Combivir (lamivudine and zidovudine, 23%), Truvada (tenofovir and emtricitabine, 17%).

CD4+ T cell count and HIV RNA

Our analyses used data from six sequential semi-annual visits that spanned a mean period of 2.5 years (range 2.1 – 2.8 years). Among HIV-infected women, mean CD4+ T cell count was 414 cells/mm^3 at the first of the six visits, which was 12 – 18 months before first use of HAART (Figure 1). Among these women, mean CD4+ T cell count was 332 cells/mm^3 at the third visit, which was the last visit prior to initiation of HAART. After HAART initiation, mean CD4+ T cell count among HIV-infected women reached a high of 479 cells/mm^3 at the sixth visit, which was 12 – 18 months after HAART initiation. Of the 127 HAART initiators, 31 failed to achieve a CD4+ T cell count above 350 cells/mm^3 at any of the three semi-annual visits after HAART initiation.

Mean $\log_{10}$ HIV RNA was 4.4 at the last visit prior to HAART initiation (Figure 1). Viral suppression to < 80 copies/ml was achieved by 43%, 58%, and 51% of women at the first, second and third semi-annual visit after initiation of HAART, respectively.

Correlation of biomarkers with clinical variables

In cross-sectional analyses, higher HIV RNA was associated with higher levels of TNF-alpha, soluble IL-2 receptor, IL-10, MCP-1 and D-dimer, both pre- and post-HAART (Supplemental Table 2). Cross-sectionally, both lower current and lower nadir CD4+ T cell count were associated with higher levels of soluble CD14, TNF-alpha, IL-6 and IL-10 both prior to and after initiating HAART; nadir CD4+ T cell count was additionally associated with pre- and post-HAART soluble IL-2 receptor levels (Supplemental Table 2). Using the longitudinal data, we found that the degree of response to HAART, as defined by changes in CD4+ T cell count and HIV RNA after treatment initiation, were significantly associated with the magnitude of changes in soluble CD14, TNF-alpha, soluble IL-2 receptor, IL-10, and MCP-1 levels (Supplemental Table 3). Age was correlated with increased soluble CD14 (only prior to HAART initiation), IL-6 and fibrinogen. Number of years smoked was correlated with increased IL-6. BMI was correlated with increased post-HAART fibrinogen levels. Presence of HCV RNA was positively correlated with soluble CD14 (only prior to HAART initiation), IL-6, IL-10, TNF-alpha (only after HAART initiation) and soluble IL-2 receptor (only after HAART initiation) (Supplemental Table 4).

Inflammation- and hemostasis-related biomarkers: Association with untreated HIV infection

As compared with HIV-uninfected women, HIV-infected women in the period prior to HAART initiation had significantly higher levels of TNF-alpha ($P < .0001$), IL-10 ($P < .0001$), soluble IL-2 receptor ($P < .0001$), soluble CD14 ($P < .0001$), MCP-1 ($P < .001$) and D-dimer ($P < 0.01$) (Figure 2). IL-6 and fibrinogen levels were not elevated among untreated HIV-infected as compared with HIV-uninfected women.

Inflammation- and hemostasis-related biomarkers: Effect of HAART initiation

After HAART initiation, levels of TNF-alpha and soluble IL-2 receptor decreased among HIV-infected women, but over the period of time after HAART had been initiated, levels of these biomarkers still remained elevated among HIV-infected women as compared with HIV-uninfected controls (Figure 2). In contrast, after initiation of HAART, levels of MCP-1 and D-dimer decreased and were no longer elevated among HAART-treated HIV-infected women relative to matched HIV-uninfected controls. IL-10 levels decreased gradually and were no longer elevated at the third semi-annual HAART-treated visit. Results for soluble CD14 varied significantly by HCV status, as described below. As compared with HIV-uninfected women, in treated HIV-infected women IL-6 levels were similar or slightly reduced, and fibrinogen levels were also similar.

Analyses of women achieving viral suppression on HAART

When we limited the analysis to 91 HAART-treated HIV-infected women who achieved HIV RNA < 80 copies/mL, TNF-alpha levels remained elevated relative to HIV-uninfected controls. Levels of TNF-alpha were 4.2 pg/mL among HIV-infected women at HAART-treated visits where viral suppression was achieved, as compared with 3.4 pg/mL among HIV-uninfected women ($P = 0.0002$). In this group of HAART-treated, aviremic women, lower CD4+ T cell count was significantly correlated with higher levels of TNF-alpha ($r = -.31$, $P = .002$).

Analyses of women with hepatitis C virus coinfection

HCV co-infection modified effects of HIV and HAART on levels of soluble CD14 ($P_{interaction} = .02$). In the subgroup of HCV-infected women, HAART initiation reduced soluble CD14 levels in the HIV-infected group, and also equalized soluble CD14 levels when comparing the HAART-treated HIV-HCV coinfected women with the HIV-uninfected, HCV-infected group (Supplemental Table 5 and Supplemental Figure 1). In contrast, soluble CD14 remained persistently elevated with HIV infection and was unaffected by HAART in the group of HCV-uninfected women. For all other biomarkers, the effects of HIV infection or HAART did not differ across HCV co-infected and non-HCV-coinfected subgroups (data not shown).

Inflammation-related and hemostasis biomarkers: Association with subclinical atherosclerosis

Among the 127 HIV-infected women who initiated HAART, 81 had a carotid artery ultrasound measurement performed subsequent to the biomarker measurements. When measured prior to HAART initiation, biomarker levels were not associated with CIMT. By contrast, when the same biomarkers were measured after initiation of HAART, greater CIMT was associated with higher levels of soluble IL-2 receptor (per 10% higher level of biomarker, difference [Δ] in CIMT = 6.0 μm, 95% CI = 1.0 – 11.0 μm, $P = .02$), IL-6 (Δ CIMT = 3.1 μm, 95% CI = −0.1 – 6.3 μm, $P = .05$), D-dimer (Δ CIMT = 3.5 μm, 95% CI = 0.4 – 6.6 μm, $P = .03$) and (of borderline significance) MCP-1 (Δ CIMT = 4.2 μm, 95% CI = −0.5 – 9.0, $P = .08$) (Table 2). Other inflammation-related and hemostasis markers measured after HAART initiation were not associated with CIMT. These analyses were adjusted for confounders including age, race/ethnicity, smoking, and BMI; further adjustment for CD4+ T cell count, HIV RNA, and antiretroviral drug class did not change the results appreciably.

Discussion

We characterized biomarkers of inflammation and hemostasis before and after first use of HAART (Table 3 summarizes key findings). As compared with HIV-uninfected controls, HIV-infected women studied in the 18 months prior to HAART initiation had increased circulating levels of the macrophage pathogen-recognition receptor CD14, several inflammation-related cytokines (TNF-alpha, IL-10), the chemokine MCP-1/CCL2, soluble IL-2 receptors, and the fibrin clot degradation marker D-dimer. Initiation of HAART tended to normalize levels of most inflammation and hemostasis biomarkers, reducing them among women using effective antiretroviral treatment to levels observed in HIV-uninfected controls. On the other hand, elevated levels of TNF-alpha persisted even in HIV-infected women who were treated with HAART and had viral suppression to below detectable limits (HIV RNA < 80 copies/mL). Finally, HIV-infected women with higher levels of IL-6, soluble IL-2 receptors, and D-dimer while on HAART had significantly higher subclinical atherosclerosis as measured by CIMT. Thus, while HAART may cause adverse metabolic disturbances, this may be balanced in effectively-treated patients by improvements in other CVD-related risk markers.

High IL-6 and D-dimer levels are well-known risk factors for CVD in individuals free of HIV infection[18] and for mortality in HIV-infected patients[2] . We extend these prior findings by linking these biomarkers with subclinical atherosclerosis (CIMT) in treated HIV-infected women. In addition, we report an association between higher soluble IL-2 receptor levels with treated HIV infection and CIMT. IL-2 is a cytokine mainly secreted by T cells; the biologic relevance of circulating soluble IL-2 receptors is uncertain. However, elevated soluble IL-2 receptors is a potential marker for T cell activation[19], and our present findings bolster prior evidence linking T cell activation in HIV seropositive individuals with preclinical vascular disease[20,21]. The association between MCP-1 levels, another biomarker that was increased in HIV-infected women, and CIMT was of borderline statistical significance, but is consistent with prior evidence suggesting a role for MCP-1 in elevated CVD risk among HIV-infected adults [22,23]. This observation may be explained by the involvement of MCP-1, which is expressed by smooth muscle cells infected by HIV[24], in transmigration of monocytes from the circulation into the subendothelium. While inflammation and coagulation markers were associated with increased CIMT among HAART-treated HIV-infected women, the same measures were not associated with CIMT when measured before the first use of HAART. Possibly, in HAART-treated patients, the presence of continued inflammation and activated coagulation despite effective HIV therapy reflects a host factor, an HIV factor, or co-factor (e.g., viral co-infection) that persistently contributes to CVD over the duration of treated HIV infection, or reflects the inflammation associated with atherosclerosis itself.

Beyond IL-6, D-dimer, soluble IL-2 receptors and MCP-1, several other inflammation-related biomarkers have been previously associated with CVD risk, even though we did not find that they predicted CIMT in our study. TNF-alpha, a pro-inflammatory cytokine expressed by cells of both the innate immune system (macrophages) and adaptive immune system (T cells), remained elevated in HIV-infected women who were using HAART and had undetectable or very low levels of circulating HIV RNA. TNF-alpha drives inflammation and apoptosis in HIV infection and probably contributes to propagation of HIV replication[25]. TNF-alpha levels predict risk of CVD events in the non—HIV-infected population[26], and might therefore be hypothesized as a CVD risk factor in untreated and treated HIV-infected populations, despite the lack of association with CIMT in our study. While several pro-inflammatory biomarkers were elevated with HIV infection, at the same time HIV infection was also associated with elevated levels of IL-10, which is an anti-inflammatory cytokine secreted by Th2 cells, T_{regs} and monocytes/macrophages. IL-10 has potential anti-atherogenic properties[27], and high IL-10 production is associated with reduced risk of stroke in non—HIV-infected populations[28]. Although no association between IL-10 and CIMT was observed in the present cohort, it remains possible that IL-10 or other inflammatory mediators invoked by HIV infection and reduced by HAART may possibly reduce CVD in certain HIV-infected individuals.

We found that circulating soluble CD14, a pathogen recognition receptor expressed by monocytes, was elevated in patients who had HIV infection, HCV infection or both. Because CD14 is produced by hepatocytes[29], it is therefore unclear whether high levels of soluble CD14 may reflect liver function, receptor shedding from activated monocytes, and/or other aspects of HIV-related and non-HIV-related disease processes. Soluble CD14 levels predict increased risk of CVD events as well as immunologic disease progression in HIV-infected populations[30,31]. Accordingly, it will be important to understand why elevated soluble CD14 levels remain elevated in HIV mono-infected and HIV – HCV coinfected individuals even with antiretroviral therapy. While no overall association between soluble CD14 and CIMT was observed, we lacked statistical power to analyze this association in HCV-infected and HCV-uninfected subgroups, which is an important limitation.

Prior evidence describing inflammation and hemostatic perturbation in HIV-infected adults are only partially consistent with the present data. Among participants in the SMART trial, elevated levels of IL-6 and D-dimer were observed in treated, well-controlled HIV-infected patients as compared with controls from external population-based cohorts[8]. In contrast, among HIV-infected women in the present study, IL-6 levels were not elevated in either the untreated or the treated phase of HIV infection, and D-dimer levels were similar between HAART-treated HIV-infected women and HIV-uninfected women. However, we did find an association in post-HAART levels of both biomarkers with greater CIMT, supporting the conclusions from the SMART study that these are important markers of vascular risk. It is important to note that our WIHS cohort was limited to women, while several studies have highlighted possible biomarker differences by gender [8,10,32].

Given the nature of the WIHS cohort, caution should be used when generalizing the results and making comparisons with previously published reports. The female WIHS cohort participants are predominantly African-American and Latina and have low socioeconomic status and a high burden of obesity, poor oral health[33], and smoking. These cofactors may interact with HIV infection to promote inflammation and aberrations in coagulation markers. Levels of several biomarkers were high in the HIV-infected and HIV-uninfected WIHS participants as compared with previously described comparison populations[8,26,34]. Many WIHS participants have impaired liver function due to hepatitis infection, medications or obesity. Our results agree with prior evidence that liver function has an important role in influencing levels of many circulating inflammation and hemostasis-related biomarkers[8,32]. Future studies are needed to address the additional contributions of factors such as liver fibrosis, renal dysfunction, diabetes and hypercholesterolemia, which were not measured in the present study. In addition, WIHS is a cohort study where selection of antiretroviral medication treatment strategies were chosen by physicians, rather than determined by a study protocol. It should be noted that this study period includes use of several older antiretroviral drugs, which could both be less effective and more metabolically toxic than current drugs[35,36], and might therefore have contributed to the non-normalization of biomarker levels among HAART-treated HIV-infected women. Finally, CIMT is a well-established measure of atherosclerosis but may not fully capture the effect of HIV on risk of cardiovascular events.

In summary, our findings confirm and extend current concepts about immune, inflammatory and hemostatic responses that are invoked by chronic HIV infection. These perturbations are observed even in the context of effective antiretroviral treatment. Additionally, higher post-HAART levels of certain inflammatory and hemostatic biomarkers were associated with increased subclinical CVD. Further investigation of inflammation and hemostatic mediators might identify pathways that can be targeted to ameliorate the long-term complications of HIV infection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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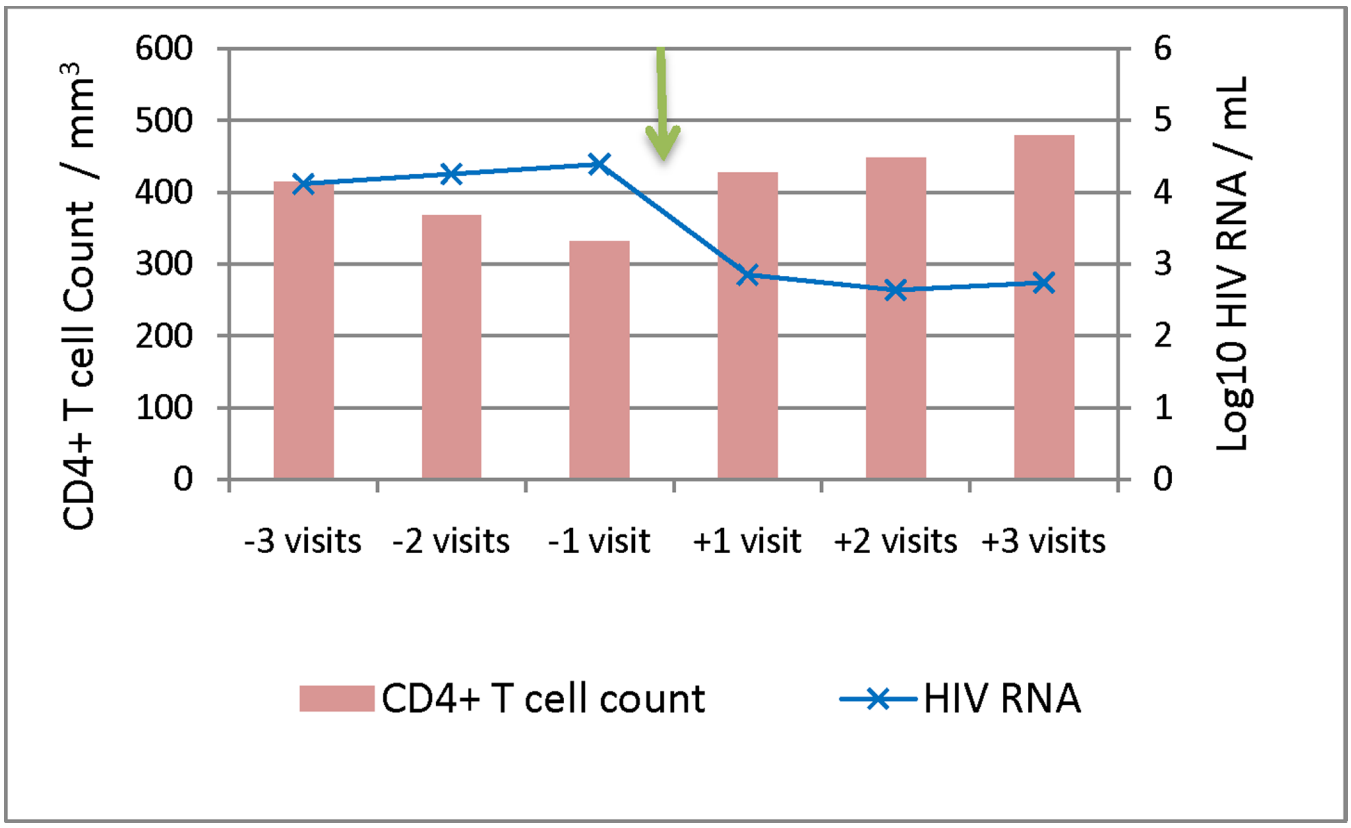
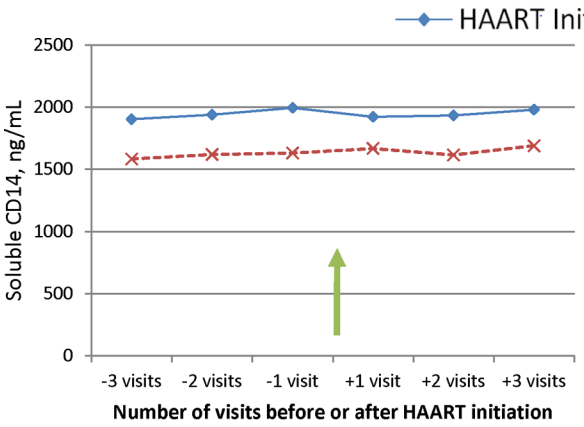
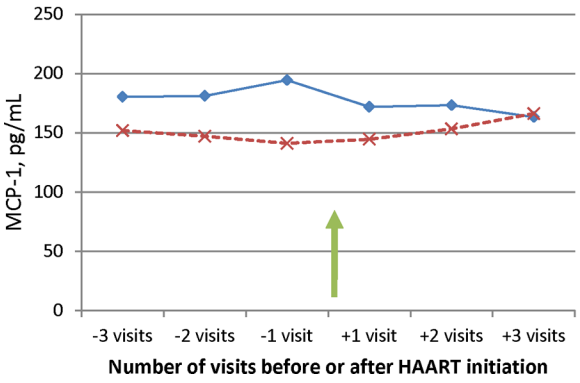


Figure 1. CD4+ T cell count and HIV RNA among HIV-infected women who initiated highly-active antiretroviral therapy

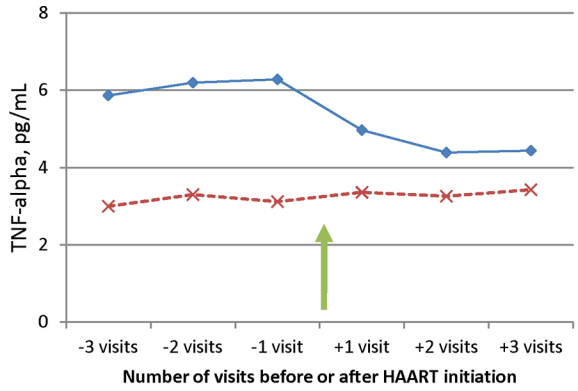
Measurements were performed at six sequential study examinations, conducted approximately six months apart. Mean values are shown. Green arrow indicates time of HAART initiation.



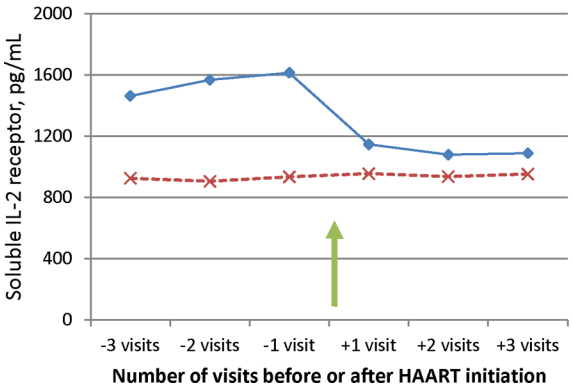
	Median sCD14, ng/mL	IQR	*P (vs. HIV-)*	*P (vs. Pre-HAART)*
HIV-	1662	(1444-1990)	N/A	N/A
Pre-HAART	1945	(1653-2315)	<0.0001	N/A
1st Post-HAART	1924	(1659-2230)	<0.0001	0.18
2nd Post-HAART	1934	(1642-2327)	<0.0001	0.71
3rd Post-HAART	1982	(1646-2248)	<0.0001	0.23



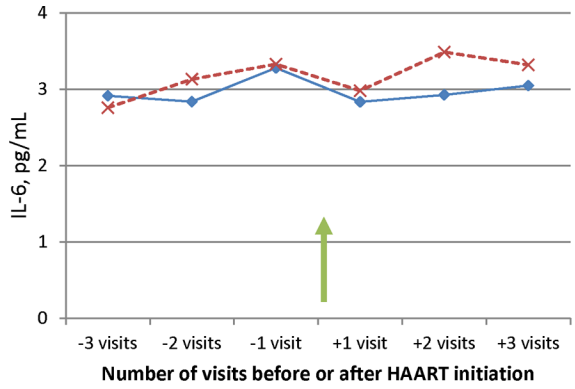
	Median MCP-1, pg/mL	IQR	*P (vs. HIV-)*	*P (vs. Pre-HAART)*
HIV-	163	(122-211)	N/A	N/A
Pre-HAART	190	(137-251)	<0.001	N/A
1st Post-HAART	172	(120-250)	0.05	<0.01
2nd Post-HAART	173	(125-251)	0.09	0.03
3rd Post-HAART	163	(117-235)	0.31	<0.01



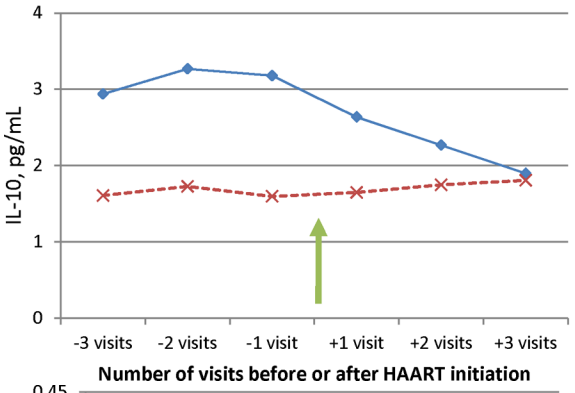
	Median TNF-α, pg/mL	IQR	*P (vs. HIV-)*	*P (vs. Pre-HAART)*
HIV-	3.4	(2.6-4.6)	N/A	N/A
Pre-HAART	6.3	(4.7-8.1)	<0.0001	N/A
1st Post-HAART	5.0	(3.4-7.1)	<0.0001	<0.0001
2nd Post-HAART	4.4	(3.2-6.7)	<0.0001	<0.0001
3rd Post-HAART	4.4	(2.9-5.9)	<0.001	<0.0001



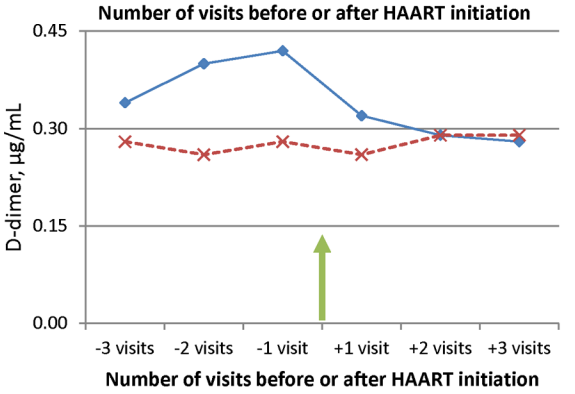
	Median IL-2sr, pg/mL	IQR	*P (vs. HIV-)*	*P (vs. Pre-HAART)*
HIV-	949	(804-1162)	N/A	N/A
Pre-HAART	1587	(1192-2107)	<0.0001	N/A
1st Post-HAART	1147	(916-1573)	<0.0001	<0.0001
2nd Post-HAART	1080	(819-1506)	<0.01	<0.0001
3rd Post-HAART	1089	(845-1558)	<0.01	<0.0001



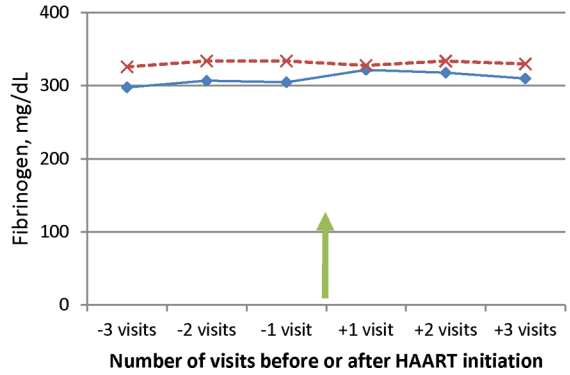
	Median IL-6, pg/mL	IQR	*P (vs. HIV-)*	*P (vs. Pre-HAART)*
HIV-	4.6	(2.0-7.1)	N/A	N/A
Pre-HAART	3.6	(2.1-5.4)	0.08	N/A
1st Post-HAART	2.8	(1.8-5.3)	<0.01	0.06
2nd Post-HAART	2.9	(1.7-6.1)	0.06	0.05
3rd Post-HAART	3.1	(1.9-4.9)	<0.01	<0.01



	Median IL-10, pg/mL	IQR	*P (vs. HIV-)*	*P (vs. Pre-HAART)*
HIV-	1.9	(1.3-2.5)	N/A	N/A
Pre-HAART	3.3	(2.2-5.2)	<0.0001	N/A
1st Post-HAART	2.6	(1.7-4.2)	<0.0001	<0.01
2nd Post-HAART	2.3	(1.6-3.3)	<0.01	<0.0001
3rd Post-HAART	1.9	(1.3-3.3)	0.06	<0.0001



	Median D-dimer, µg/mL	IQR	*P (vs. HIV-)*	*P (vs. Pre-HAART)*
HIV-	0.31	(0.23-0.47)	N/A	N/A
Pre-HAART	0.43	(0.26-0.69)	<0.01	N/A
1st Post-HAART	0.32	(0.24-0.62)	0.13	0.07
2nd Post-HAART	0.29	(0.21-0.45)	0.27	<0.0001
3rd Post-HAART	0.28	(0.21-0.42)	0.12	<0.0001



	Median fibrinogen, mg/dL	IQR	*P (vs. HIV-)*	*P (vs. Pre-HAART)*
HIV-	334	(262-379)	N/A	N/A
Pre-HAART	310	(267-349)	0.07	N/A
1st Post-HAART	322	(267-372)	0.36	0.15
2nd Post-HAART	318	(254-383)	0.18	0.67
3rd Post-HAART	310	(253-373)	0.10	0.88

Figure 2. Changes in circulating inflammation and hemostasis biomarkers among 127 HIV-infected women initiating HAART and matched HIV-uninfected controls

HIV-infected subjects included women under study observation both prior to treatment, and after first exposure to HAART. HIV-uninfected women were individually matched to HAART initiators by calendar time, age, race, body mass index, smoking, and hepatitis C infection. At six sequential study examinations, conducted approximately six months apart, measurements were performed of soluble CD14 (sCD14), monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor-alpha (TNF-α), soluble interleukin-2 receptor (IL-2sr), IL-6, IL-10, D-dimer and fibrinogen. Green arrow indicates time of initiation of highly-active antiretroviral therapy (HAART). Data shown are medians. Medians and interquartile ranges (IQRs) of biomarkers are presented for HIV-uninfected women (mean of six sequential visits), pre-HAART (mean of 3 pre-HAART visits) and each individual post-HAART visit. *P* values were calculated using the Wilcoxon signed rank test.

Table 1

Characteristics of HIV-infected women initiating HAART and matched HIV-uninfected control women.

	HIV-infected women (n=127)	HIV-uninfected women (n=127)	
	% or median (IQR)	% or median (IQR)	*P**
Age, years	37 (33–42)	37 (31–43)	.99
Race and ethnicity			.94
African-American	59%	61%	
Latina	24%	24%	
White/Other	17%	15%	
Current smoking	53%	54%	.85
Body mass index			.52
Underweight (< 18.5)	2%	3%	
Normal (18.5 – 25)	36%	30%	
Overweight (25 – 30)	33%	31%	
Obese (> 30)	28%	35%	
HCV antibody positive	30%	31%	.89
Calendar year	1999 (1997–2004)	1999 (1997–2003)	.59
PI use	53%	N/A	
NNRTI use	35%	N/A	
NRTI use	93%	N/A	

* P values calculated using Chi square or Mann-Whitney test as appropriate

All characteristics displayed in the table were matching variables. For the purpose of matching, we used age, body mass index and smoking status assessments that were collected at the fourth in the series of six consecutive visits (representing the first visit after HAART initiation among HIV-infected women, and the corresponding calendar time-matched visit among HIV-uninfected women). Baseline HCV antibody status was used for matching. The mean (range) propensity score for both the HIV-uninfected and HIV-infected groups was .07 (<.01 – .24); a tolerance of 5% was used when selecting matched HIV-infected and HIV-uninfected pairs. Body mass index was calculated as the weight in kilograms divided by the square of the height in meters.

HCV, hepatitis C virus; IQR, interquartile range; PI, protease inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor.

Table 2

Associations of inflammation-related and hemostasis biomarkers with carotid artery intima-media thickness (CIMT) among 81 HIV-infected women, before and after initiating highly active antiretroviral therapy (HAART)

	Prior to HAART initiation Difference in CIMT in μm per 10% increase in biomarker			After HAART initiation Difference in CIMT in μm per 10% increase in biomarker		
	Effect estimate	95% CI	*P*	Effect estimate	95% CI	*P*
Soluble CD14, ng/mL	−2.9	−11.6 , 5.8	0.51	−4.2	−12.4 , 4	0.31
Tumor necrosis factor-alpha, pg/mL	−1.6	−6.3 , 3.1	0.5	2.4	−0.9 , 5.7	0.15
Soluble IL-2 receptor, pg/mL	3.3	−1.3 , 8	0.16	6	1 , 11	0.02
IL-6, pg/mL	0.2	−2.9 , 3.2	0.92	3.1	−0.1 , 6.3	0.05
IL-10, pg/mL	−2	−5.4 , 1.4	0.25	1.3	−1.7 , 4.2	0.4
MCP-1, pg/mL	1.7	−2.8 , 6.2	0.46	4.2	−0.5 , 9	0.08
D-dimer, μg/mL	−0.3	−3.3 , 2.7	0.83	3.5	0.4 , 6.6	0.03
Fibrinogen, mg/dL	−6.8	−17.3 , 3.6	0.2	0.1	−9.4 , 9.7	0.98

Table 3

Summary: Associations of inflammation and hemostasis biomarkers with HIV infection and initiation of highly active antiretroviral therapy

	Pre-HAART HIV infection visits *versus* HIV-uninfected	Post-HAART HIV infection visits *versus* HIV-uninfected	Treated, aviremic HIV infection visits *versus* HIV-uninfected	Biomarker associated with carotid artery intima-media thickness (*P* < .05)?
Soluble CD14	↑	*	*	No
TNF-alpha	↑	↑	↑	No
Soluble IL-2 receptor	↑	↑	-	Yes
IL-6	-	↓	-	Yes
IL-10	↑	-	-	No
MCP-1	↑	-	-	No
D-dimer	↑	-	-	Yes
Fibrinogen	-	-	-	No

↑, increased in HIV-infected women as compared with HIV-uninfected controls;

↓, decreased in HIV-infected women as compared with HIV-uninfected controls

*Associations of soluble CD14 with HAART-treated HIV infection differed significantly by HCV infection status, therefore no overall summary of the findings is presented here.

HAART, highly active antiretroviral therapy